

CLAIMS

1. An isolated EBV CTL peptide epitope comprising at least nine contiguous amino acid residues of an LMP1 protein, wherein said EBV CTL peptide is not YLQQNWWTL (SEQ ID NO:1); ESDSNSNEG (SEQ ID NO:2) or 5 YLLEMLWRL (SEQ ID NO:3).
2. The isolated EBV CTL peptide epitope of Claim 1, comprising an amino acid sequence selected from the group consisting of:
 - (i) QRH (SEQ ID NO:4);
 - (ii) AGNDG (SEQ ID NO:5);
 - 10 (iii) QNW (SEQ ID NO:6), specifically excluding the sequence YLQQNWWTL (SEQ ID NO:1);
 - (iv) VLYS (SEQ ID NO:7); and
 - (v) DSNSNE (SEQ ID NO:8), specifically excluding the amino acid sequence ESDSNSNEG (SEQ ID NO:2).
- 15 3. The isolated EBV CTL peptide epitope of Claim 2 consisting essentially of an amino acid sequence selected from the group consisting of:
 - (i) QRHSDEHHH (SEQ ID NO:9);
 - (ii) GQRHSDEHH (SEQ ID NO:10);
 - (iii) YYHGQRHSD (SEQ ID NO:11); and
 - 20 (iv) WMYYHGQRH (SEQ ID NO:12).
4. The isolated EBV CTL peptide epitope of Claim 3 consisting essentially of an amino acid sequence selected from the group consisting of:
 - (i) YYHGQRHSDEHH (SEQ ID NO:13);
 - (ii) IWMYYHGQRHSD (SEQ ID NO:14); and
 - 25 (iii) LIWMYYHGQRHSDEHHH (SEQ ID NO:15).
5. The isolated EBV CTL epitope of Claim 2 consisting essentially of an amino acid sequence selected from the group consisting of:
 - (i) AGNDGGPPQ (SEQ ID NO:16); and
 - (ii) PSDSAGNDG (SEQ ID NO:17).
- 30 6. The isolated EBV CTL epitope of Claim 5 that consisting essentially of an amino acid sequence selected from the group consisting of:
 - (i) SDSAGNDGGPPQ (SEQ ID NO:18);

- (ii) DSAGNDGGPPQL (SEQ ID NO:19); and
- (iii) PHSPSDSAGNDGGPPQL (SEQ ID NO:20).

7. The isolated EBV CTL epitope of Claim 2 consisting essentially of an amino acid sequence selected from the group consisting of:

- 5 (i) IALYLQQNW (SEQ ID NO:21);
- (ii) ALYLQQNWW (SEQ ID NO:22);
- (iii) QNWWTLLVD (SEQ ID NO:23); and
- (iv) LYLQQNWWT (SEQ ID NO:24).

8. The EBV CTL epitope of Claim 7 consisting essentially of an amino acid sequence selected from the group consisting of:

- (i) IALYLQQNWWTL(SEQ ID NO:25);
- (ii) YLQQNWWTLLVD (SEQ ID NO:26); and
- (iii) LIALYLQQNWWTLLVD (SEQ ID NO:27).

9. The EBV CTL peptide epitope of Claim 2 consisting essentially of an amino acid sequence selected from the group consisting of:

- (i) ALLVLYSFAL(SEQ ID NO:28);
- (ii) LLVLYSFAL (SEQ ID NO:29);
- (iii) ALLVLYSFA (SEQ ID NO:30); and
- (iv) VLYSFALML (SEQ ID NO:31).

20 10. The EBV CTL peptide epitope of Claim 9 consisting essentially of an amino acid sequence selected from the group consisting of:

- (i) ALLVLYSFALML (SEQ ID NO:32);
- (ii) GALLVLYSFALM (SEQ ID NO:33);
- (iii) DWTGGALLVLYS (SEQ ID NO:34);
- 25 (iv) GGALLVLYSFAL (SEQ ID NO:35); and
- (v) DWTGGALLVLYSFALML (SEQ ID NO:36).

11. The EBV CTL peptide epitope of Claim 2 consisting essentially of an amino acid sequence selected from the group consisting of:

- 30 (i) DSNSNEGRH (SEQ ID NO:37).
- (ii) SGHESDSNSNEG (SEQ ID NO:38); and
- (iii) TDDSGHESDSNSNEGRH (SEQ ID NO:39).

12. An isolated EBV CTL peptide epitope consisting essentially of an amino acid sequence selected from the group consisting of: SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:16; SEQ ID NO:17; SEQ ID NO:21; SEQ ID NO:22; SEQ ID NO:23; SEQ ID NO:24; SEQ ID NO:28; 5 SEQ ID NO:29; SEQ ID NO:30; SEQ ID NO:31; and SEQ ID NO:37
13. A variant of an isolated EBV peptide epitope having an amino acid sequence according to any one of SEQ ID NOS:40-50.
14. An isolated protein comprising at least one EBV CTL epitope according to any one of Claims 1-12.
- 10 15. The isolated protein of Claim 14 which is a polyepitope protein comprising an amino acid sequence selected from the group consisting of ALLVLYSFA (SEQ ID NO:30) and IALYQQNW (SEQ ID NO:21).
16. The isolated polyepitope protein of Claim 14 comprising thirteen EBV CTL epitopes having the respective amino acid sequences YLLEMLWRL (SEQ ID NO: 3); YLQQNWWTL (SEQ ID NO: 1); ALLVLYSFA (SEQ ID NO:30); 15 IAYLQQNW (SEQ ID NO:21); SSCSSCPLSKI (SEQ ID NO: 51); PYLFWLAAI (SEQ ID NO:52); TYGPVFMCL (SEQ ID NO:53); RRRWRRLLTV (SEQ ID NO:54); LLSAWILTA (SEQ ID NO: 55); LTAGFLIFL (SEQ ID NO:56); VMSNTLLSAW (SEQ ID NO:57); IEDPPFNSL (SEQ ID NO:58); CLGGLLTMV (SEQ ID NO:59).
17. The isolated polyepitope protein of Claim 15 comprising the amino acid sequence set forth in SEQ ID NO:81.
18. An isolated nucleic acid encoding the isolated EBV CTL epitope of any one of Claims 1-12.
- 25 19. An isolated nucleic acid encoding the isolated protein of any one of Claims 14-17.
20. An isolated nucleic acid encoding the variant EBV peptide epitope of Claim 13.
21. The isolated nucleic acid of Claim 20 comprising a nucleotide sequence as 30 set forth in any one of SEQ ID NOS: 63-65, 67-69, 71-76 or 78-80.

22. The isolated nucleic acid of Claim 19 which comprises the nucleotide sequence set forth in SEQ ID NO:80.
23. An expression construct comprising the isolated nucleic acid of any one of Claims 18-22 operably linked to one or more regulatory nucleotide sequences in
5 an expression vector.
24. The expression construct of Claim 23, which is adenovirus-based.
25. The expression construct of Claim 24, which encodes the amino acid sequence set forth in SEQ ID NO: 81.
26. A host cell or organism comprising the expression construct of Claim 23.
- 10 27. A pharmaceutical composition comprising at least one isolated EBV CTL peptide epitope according to any one of Claims 1-12 together with a pharmaceutically acceptable carrier, diluent or excipient.
28. The pharmaceutical composition of Claim 27 comprising an amino acid sequence selected from the group consisting of ALLVLYSFA (SEQ ID NO:30) and IALYQQNW (SEQ ID NO:21).
- 15 29. The pharmaceutical composition of Claim 27 comprising a polyepitope protein that comprises the amino acid sequence set forth in SEQ ID NO:81.
30. A pharmaceutical composition comprising the expression construct of Claim 23 together with a pharmaceutically acceptable carrier, diluent or excipient.
- 20 31. The pharmaceutical composition of Claim 30 comprising an expression construct that encodes an amino acid sequence selected from the group consisting of ALLVLYSFA (SEQ ID NO:30) and IALYQQNW (SEQ ID NO:21).
32. The pharmaceutical composition of Claim 29 comprising an expression construct that encodes a polyepitope protein having the amino acid sequence set
25 forth in SEQ ID NO:81.
33. The pharmaceutical composition of Claim 32 comprising the nucleotide sequence set forth in SEQ ID NO:82.
34. The pharmaceutical composition of any one of Claims 27-33, which is an immunotherapeutic composition.
- 30 35. The pharmaceutical composition of Claim 34, which is a vaccine.

36. A method of therapeutically and/or prophylactically treating an EBV-associated disease, including the step of administering to an animal at least one isolated EBV CTL epitope according to any one of Claims 1-12.
37. The method of Claim 36 wherein the at least one epitope comprises an amino acid sequence selected from the group consisting of ALLVLYSFA (SEQ ID NO:30) and IALYQQNW (SEQ ID NO:21).
38. The method of Claim 35 wherein the at least one peptide epitope is a polyepitope protein that comprises the amino acid sequence set forth in SEQ ID NO:81.
39. A method of therapeutically and/or prophylactically treating an EBV-associated disease, including the step of administering to an animal the expression construct of Claim 23.
40. The method of Claim 39 wherein the expression construct encodes a polyepitope protein that comprises an amino acid sequence selected from the group consisting of ALLVLYSFA (SEQ ID NO:30) and IALYQQNW (SEQ ID NO:21).
41. The method of Claim 40 wherein expression construct comprises the nucleotide sequence set forth in SEQ ID NO:82.
42. The method of any one of Claims 36 to 41, wherein the EBV associated disease is selected from B and T cell non-Hodgkin's lymphomas, Hodgkin's disease, and lymphoepithelioma-like carcinomas.
43. The method of Claim 42, wherein the EBV associated disease is nasopharyngeal carcinoma (NPC).
44. The method of any one of Claims 36-43 wherein the animal is a mammal.
45. The method of Claim 44 wherein the mammal is a human.
46. The method of Claim 45 wherein one or more of the at least one EBV peptide epitopes is selected according to a HLA type of the human to be treated.
47. An antibody which binds an EBV CTL epitope according to any one of Claims 1-12 or the variant of Claim 13.
48. A method of determining whether an animal harbours, or has been exposed to, Epstein Barr Virus, said method including the step of contacting one or more T cells isolated from said individual with at least one EBV peptide

epitope according to any one of Claims 1-12, whereby a response to the at least one EBV peptide epitopes by said one or more T cells indicates that the animal harbours, or has been exposed to, Epstein Barr Virus.

49. The method of Claim 48 wherein the animal is a mammal.
- 5 50. The method of Claim 49, wherein the animal is a human.
51. A method of identifying an EBV CTL epitope, said method including the steps of:
 - (i) producing a plurality of different peptides derived from an EBV LMP1 protein;
 - 10 (ii) combining said one or more of said peptides with one or more T lymphocytes obtained from an EBV seropositive individual; and
 - (iii) measuring IFN- γ production by said one or more T lymphocytes in response to said one or more peptides, wherein production of IFN- γ above a reference amount is indicative of said one or more peptides having at least one EBV CTL epitope.
- 15 52. The method of Claim 51 further including the step (iv) of determining whether said one or more T lymphocytes produced at step (ii) lyses one or more EBV-infected target cells.
53. An isolated EBV CTL epitope when obtained by the method of Claim 52.
- 20 54. The isolated CTL epitope of Claim 53 which has an amino acid sequence selected from the group consisting of: SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:16; SEQ ID NO:17; SEQ ID NO:21; SEQ ID NO:22; SEQ ID NO:23; SEQ ID NO:24; SEQ ID NO:28; SEQ ID NO:29; SEQ ID NO:30; SEQ ID NO:31; and SEQ ID NO:37.